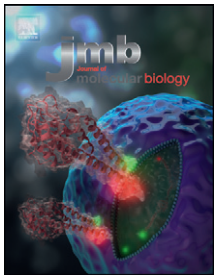

Deciphering Protein Stability in Cells

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Proteins have evolved to fold and function in environments quite different from the dilute solutions often used in laboratory experiments. Cells are crowded environments containing >200 mg/ml of biomolecules, and even extracellular environments such as plasma can contain 80 mg/ml of protein [1]. These conditions can affect protein stability and conformational distributions and promote quinary structure, transient interactions between biomolecules, that is, stickiness [2–6].

In recent years, a number of methods including NMR [7], Raman microscopy [8] and fluorescence microscopy have been adapted to monitor protein structure, stability, synthesis and/or folding in cells. One method, FRet (*f*ast *re*laxation *i*maging) developed by Gruebele and colleagues, allows real-time measurements of protein thermal stability and folding kinetics in living cells with high spatial resolution [9–11]. This is accomplished by combining in-cell measurements of Förster resonance energy transfer with small, transient temperature jumps. The workhorse protein for these experiments has been yeast PGK (*p*hospho*g*lycerate *k*inase), a cytoplasmic enzyme in the glycolysis pathway with the Förster resonance energy transfer pair provided by the donor, green fluorescent protein, AcGFP1 at the N-terminus and the acceptor, red fluorescent protein, mCherry at the C-terminus. In this issue, Guzman *et al.* apply in-cell FRet to the variable surface antigen protein VlsE from the spirochete responsible for Lyme disease *Borrelia burgdorferi* [12].

Combinations of *in vitro* experiments and *in silico* coarse-grained computational modeling show that both PGK and VlsE assume more compact conformations in crowded solutions [13,14]. For PGK, the two domains come closer together leading to increased activity, while the ellipsoidal, α-helical bundle that forms VlsE curves to become more crescent or bean-shaped. As might be expected, the in-cell conformational distributions of PGK and VlsE, as monitored by FRet, are also different from those in dilute solution. The conformational distribution of PGK in cells resembles that of PGK in crowded solutions [10] while the conformational distribution of VlsE is more heterogeneous with donor-to-acceptor ratios more consistent with the crowding-associated crescent shape than a stretched out ellipsoid [12]. Similarly, in-cell hydrogen–deuterium exchange NMR experiments show more exchange for ubiquitin in the cell than for ubiquitin in solution indicating changes in protein dynamics and/or protein conformation that may arise from quinary interactions [15]. The more compact conformations of PGK and VlsE are also consistent with theoretical results predicting that increases in excluded volume, due to the space taken by the crowding agents, should favor compaction [1,16].

In contrast, the thermal stability of PGK is increased in human osteosarcoma U2OS cells [9,10] while that of VlsE is decreased [12]. VlsE is destabilized in these cells despite the increased stability observed for VlsE in solutions containing 150 mg/ml Ficoll 70, a hard-sphere crowding agent. Similar results where changes in enthalpic and entropic contributions to protein stability are different for different types of crowders have been observed by Wang *et al.* and Sukenik *et al.* [17,18]. In particular, chymotrypsin inhibitor 2 has decreased stability in the presence of 100 mg/ml lysozyme or bovine serum albumin [19]. Similarly, molecular dynamics simulations and NMR experiments on mixtures of villin headpiece and the B1 segment of streptococcal protein G revealed that protein crowding destabilizes the villin headpiece [20]. Other proteins are also destabilized in cells [21,22]; for example, Schlesinger *et al.* have

shown that a destabilized version of Protein L can fold *in vitro* in the presence of 300 mM NaCl but is unfolded in *Escherichia coli* cells even under hyperosmotic conditions [21].

Why are some proteins destabilized in cells and/or in the presence of protein crowders? As suggested by Pielak, Feig and others, weak quinary interactions may effect protein stability by altering the conformational distribution of proteins in cells [4,15,17,19,20,23]. In addition, Guzman *et al.* point out that VlsE, which is destabilized in U2OS cells, evolved to function on the surface of *B. burgdorferi* in plasma (~80 mg/ml protein), a less crowded and sticky environment than the cytoplasm, thus suggesting that the physiological environment in which a protein has evolved is likely to affect its in-cell stability [12]. VlsE is also exported to the cell surface through the Sec pathway via at least partially unfolded intermediates [24], and the need for translocation may also favor lower in-cell stability. However, the simple need for translocation (e.g., out of the cell or to a cellular organelle) does not mean that a protein will necessarily be unfolded in the cytoplasm. Recent in-cell NMR studies of the mitochondrial protein Mia40 show that, while it must be unfolded for translocation into the mitochondrial intermembrane space, it is folded in the cytoplasm when overexpressed [25]. Other possible correlates of in-cell stability include protein turnover rates and the environment of the organism in which the protein evolved including pH, temperature, salt concentration and so on. Finally, most proteins are only marginally stable and very high protein stability may be a selective disadvantage because it can interfere with protein function and turnover [26]. All of these factors are likely to be important for in-cell stability.

The in-cell FRel experiments on PGK and VlsE highlight the effects of crowding environments on different proteins, supporting the role of the physiologically relevant environment in determining in-cell protein stability. Clearly, more proteins with different folds and from different environments must be studied in cells to fully understand these effects. Experiments by the Gruebele group and others are beginning to tease out the relative roles of physiological environment, quinary interactions, protein localization, protein lifetime and other factors that help shape the in-cell energy landscape of proteins determining in-cell conformational distributions, stabilities and folding kinetics.

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